

# Assessing the Allergic Potential of Indoor Air Fungal Contaminants

Part of a Cross-Laboratory Study: Children's Health Problems Associated with Fungal Exposure

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**SUSCEPTIBILITY,  
ALLERGY & ASTHMA**

## INTRODUCTION

### ENVIRONMENTAL ISSUE:

- Dampness in indoor environments contributes to fungal growth in both home and workplace buildings.
- Fungi are the principal microorganisms responsible for the health problems in such buildings.
- Children spend >90% of their time indoors including home, school, daycare.
- Indoor exposures of children to molds results in complex and controversial health effects.
- The toxigenic fungi *Stachybotrys chartarum* was chosen as prototype mold for these studies.

### SCIENTIFIC APPROACH:

The cross-laboratory approach provides a multi-faceted assessment and evaluation of exposure and health effects of exposure to indoor molds by using a combination of analytical, experimental, epidemiological, and modeling approaches.

### PARTICIPATING LABORATORIES AND CENTERS:

NATIONAL EXPOSURE RESEARCH LABORATORY

NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY

#### Experimental Toxicology Division:

- Establish a mouse model of allergic lung disease to *S. chartarum*.
- Identify, characterize and compare IgE inducing proteins from three fungi (*Metarhizium anisopliae*, *S. chartarum*, and *Penicillium chrysogenum*).
- Demonstrate parallels between human and rodent responses

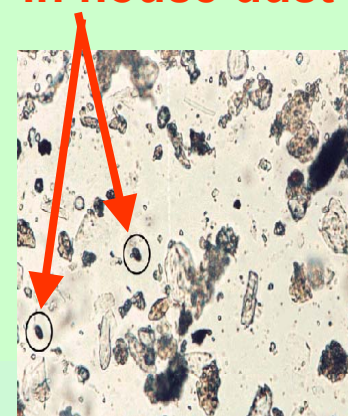
NATIONAL RISK MANAGEMENT RESEARCH LABORATORY

NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT

Mold contaminated wall board



*S. chartarum* spores in house dust



## METHODS

### Animals

Fifty day-old BALB/c mice (Charles River Laboratories, Raleigh, NC)

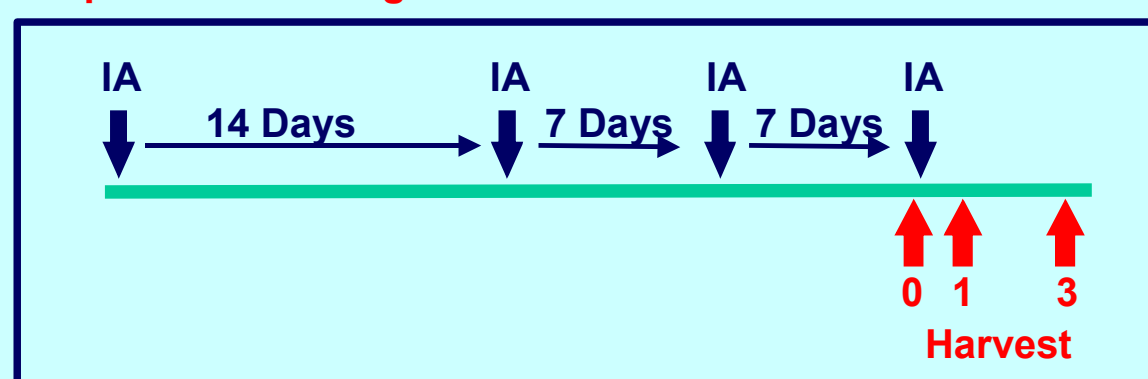
### Treatment

Fungus was ground with a mortar and pestle, stirred overnight, clarified by centrifugation, sterile filtered, and concentrated to yield a crude extract:

- MACA - equal protein amounts of *M. anisopliae* mycellial, conidial, inducible protease/chitinase extracts
- SCE-1 - a mixture of 5 *S. chartarum* isolates
- Mice received one of the following treatments by involuntary aspiration (IA) on the schedule described:
  - 4 doses of MACA or SCE-1 in HBSS
  - 4 doses of HBSS (vehicle control)
  - 4 doses of BSA in HBSS (negative control)
  - 3 doses of HBSS and 1 dose of MACA or SCE-1 (inflammatory control)

Mice were killed on day 1 and day 3 by an overdose of sodium pentobarbital and exsanguinated by cardiac puncture.

### Experimental Design



### Procedures

#### Respiratory Physiology

- Immediate respiratory physiological responses were measured after each aspiration (D-28,D-14,D-7,D0) by barometric whole-body plethysmography (Buxco system).
- Airway hyperresponsiveness was measured on day 1 and 3 by exposure to doubling concentrations of nebulized methacholine (Buxco system).

#### Antibody and cytokine ELISAs

Antibody and cytokine levels were quantified by ELISA.

#### Histopathology

Lungs were fixed in 10% buffered formalin acetate, embedded in paraffin, and sectioned. Sections were examined and scored by a board-certified veterinary pathologist (Veritas Laboratories, Inc., Burlington, NC).

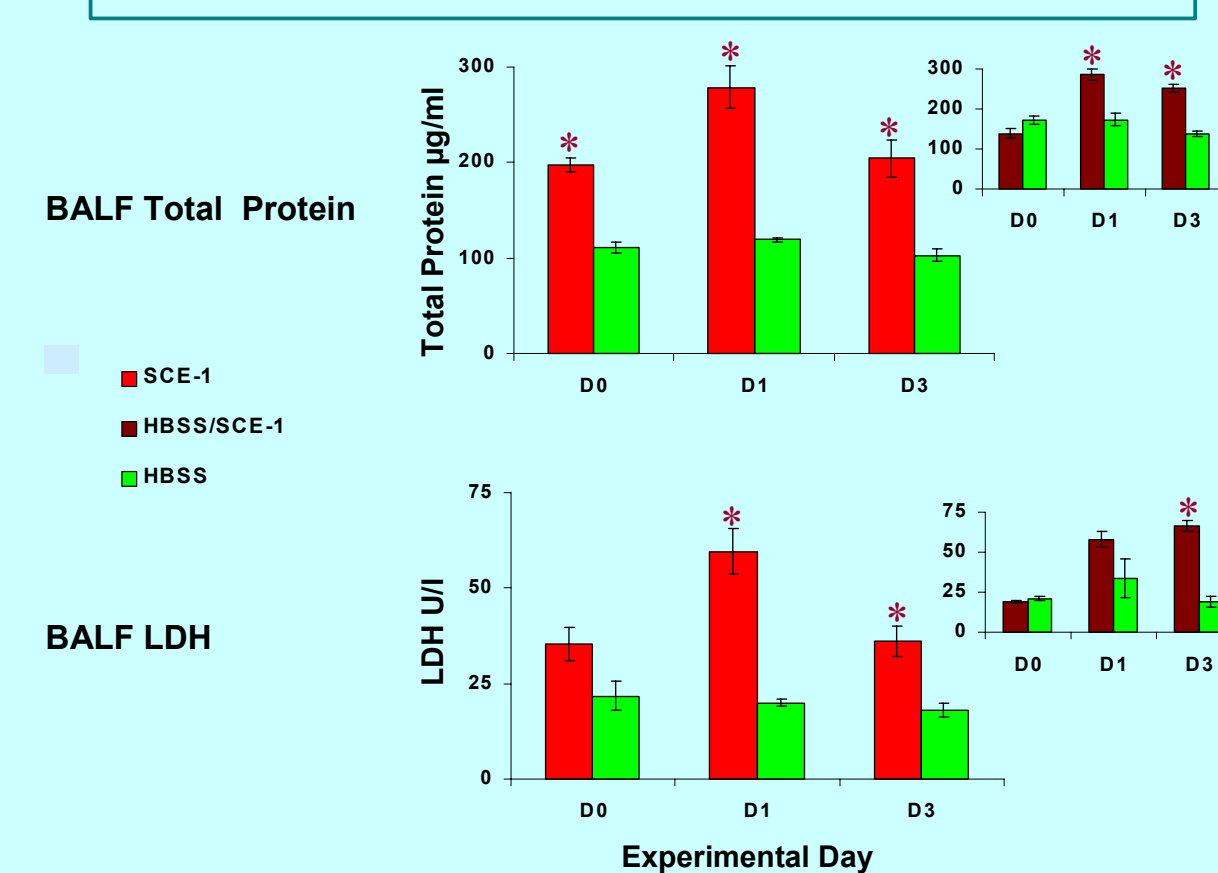
#### Statistics

Data were analyzed for statistical significance with SAS (SAS Corporation, Cary, NC), using a one-way analysis of variance.

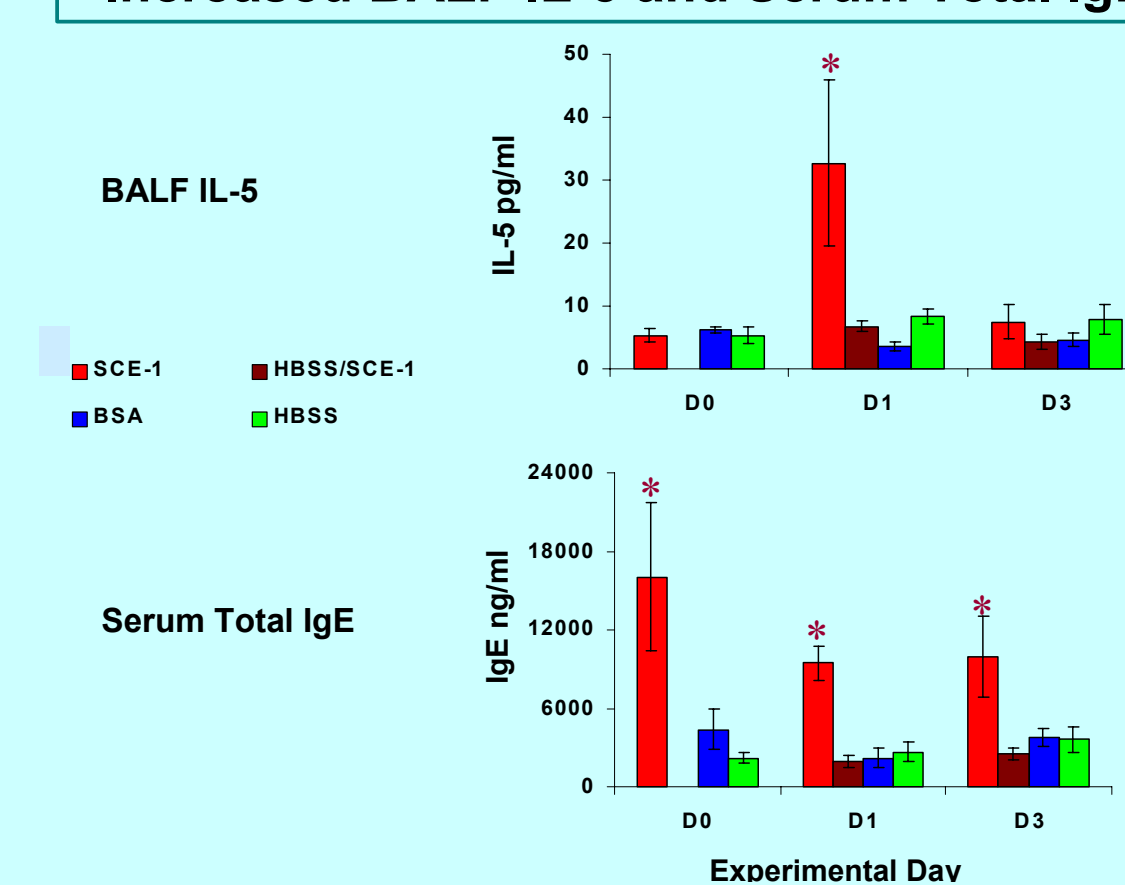
## RESULTS

Representative examples from *Stachybotrys chartarum* (SCE-1) and *Metarhizium anisopliae* (MACA) studies are presented

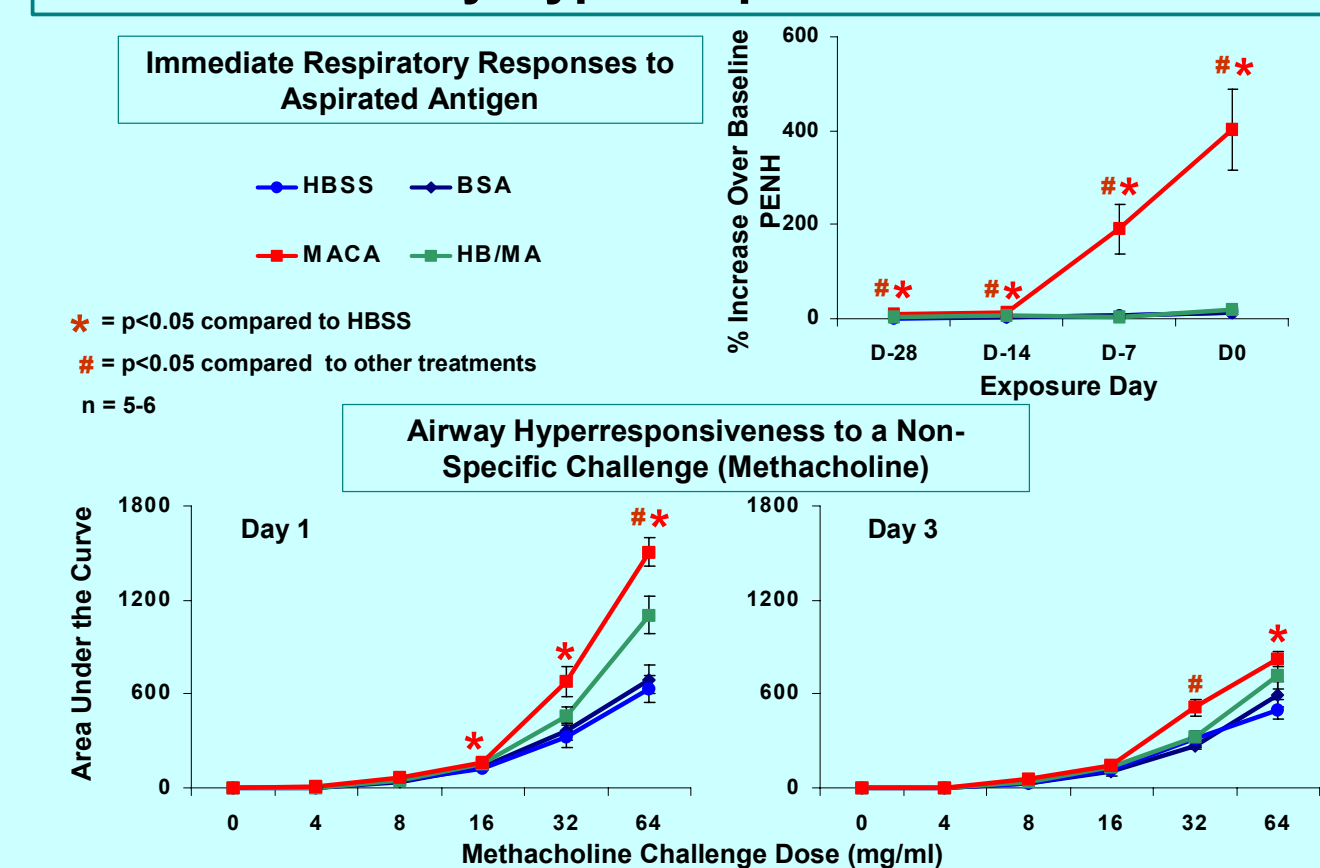
### The First Exposure to Fungal Extract Increased BALF Total Protein and LDH



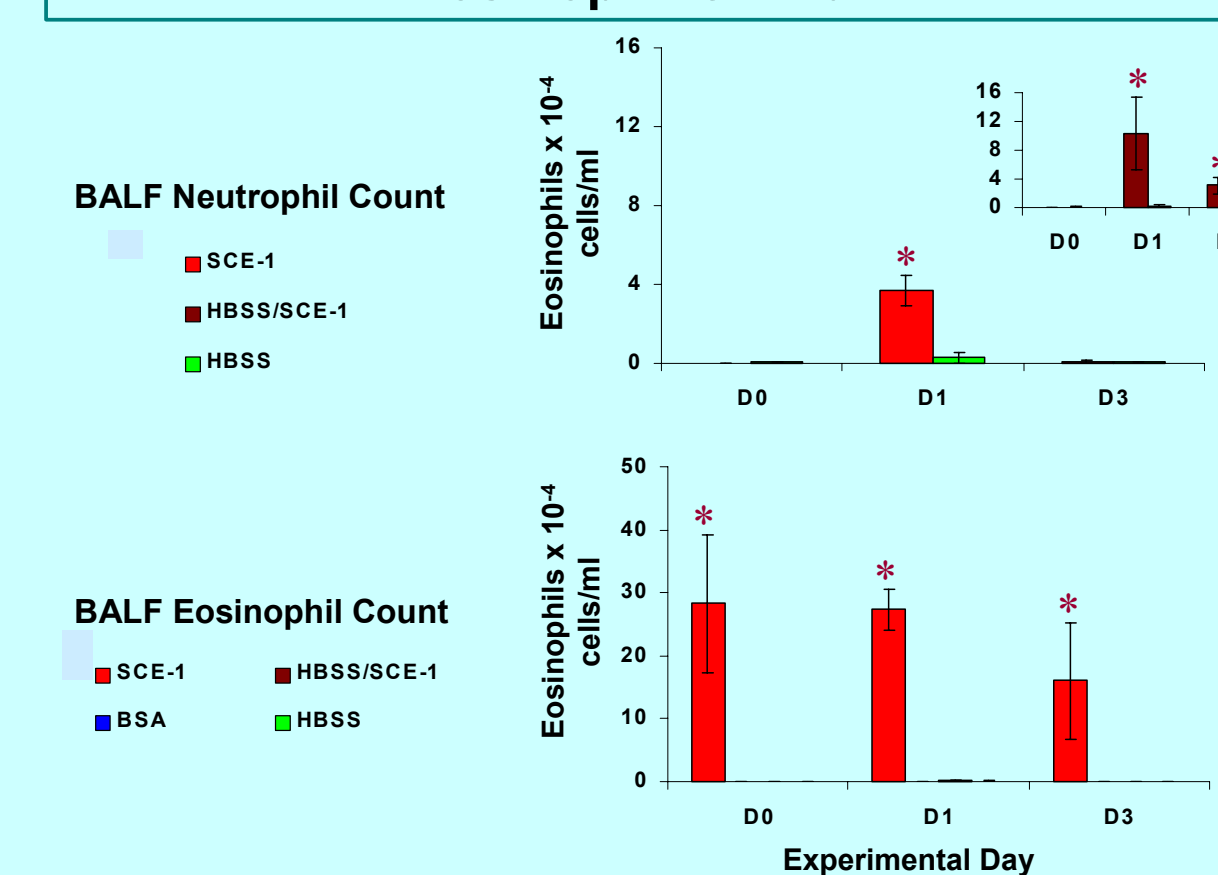
### Multiple Exposures to Fungal Extract Increased BALF IL-5 and Serum Total IgE



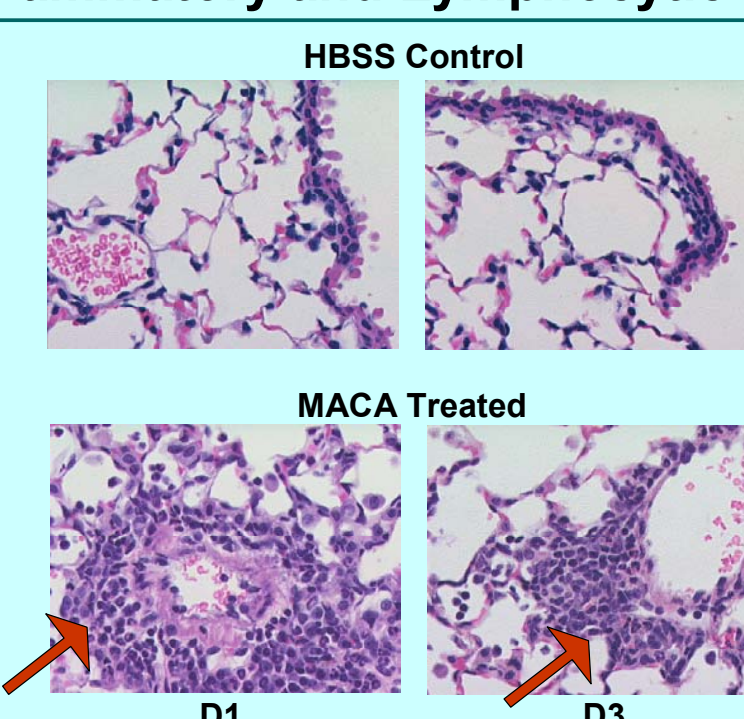
### Multiple Exposures to MACA Resulted in Increased Immediate Respiratory Responses and Airway Hyperresponsiveness



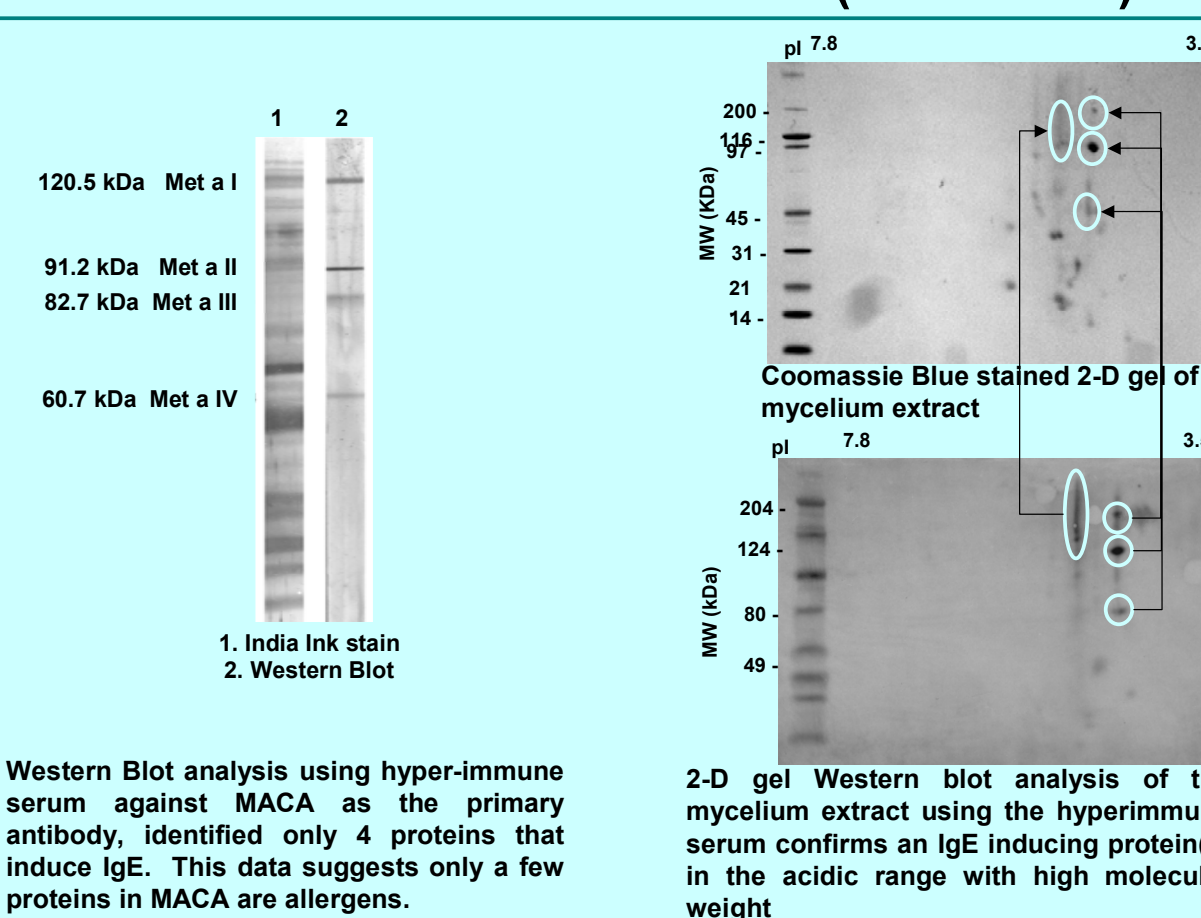
### Multiple Exposures to Fungal Extract Increased BALF Neutrophilic and Eosinophilic Influx



### Multiple Exposures to Fungal Extract Increased Lung Influx of Inflammatory and Lymphocytic Cells



### Several IgE Inducing Proteins Have Been Identified in MACA and SCE-1 (not shown)



## CONCLUSION

- Repeated pulmonary exposures to either *Stachybotrys chartarum* (SCE-1) and *Metarhizium anisopliae* (MACA) Extracts Induced:
  - Non-specific lung injury
    - Permeability - BALF total protein
    - Cell damage - BALF LDH activity
    - Neutrophilic infiltration
  - Increased antigen specific immune responses
    - Increased BALF IL-5 and eosinophilic infiltration
    - Serum total IgE
    - Immediate airway reactivity (extract exposure) and hyperresponsiveness to non-specific challenge (methacholine)
- Both of these fungal extracts have demonstrated the ability to induce responses in the mouse characteristic of allergic lung disease in humans.
- The mouse model provides the means to
  - Identify and characterize the specific allergens
  - Assess relative potency compared to other indoor allergens.

## IMPACT

- The data generated
  - Establishes methodology leading to risk assessment for children/infants from exposure to *S. chartarum* specifically but serves as a model for assessment of other indoor biological contaminants.
- Provides comprehensive information to be used by the Office of Air and Radiation and by the Regional Offices in helping our clients, the American parent, on dealing with this major environmental problem.

## FUTURE DIRECTIONS

- Continue identification and characterization of IgE inducing proteins from three fungi in collaboration with NERL scientists.
- Demonstrate parallels between human and rodent responses
- Determine relative potency of the IgE inducing proteins compared
  - Among the fungal "allergens"
  - To better characterized allergens, i.e. house dust mite, cockroach, alcalase (benchmarked detergent enzyme)

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**SOLVING AGENCY PROBLEMS**

